# The hidden resistome of retail chicken meat

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# The hidden resistome of retail chicken meat

Sir,

The increasing spread of multidrug-resistant bacteria is a worldwide health issue. The role of the environment in dissemination of resistance genes and the ultimate burden on the clinic is widely unknown [1]. Many resistance genes originate from the environment, such as *bla*$_{CTX-M}$, the most common clinical extended-spectrum β-lactamase (ESBL) conferring resistance to the important third-generation cephalosporin (3GC) antibiotics [2]. The environment also provides a potential risk to humans through direct exposure to antibiotic-resistant bacteria [1].

Despite the importance of the environment as a reservoir of diverse resistance genes, the exposure route to humans is understudied. A probable route is through the food chain, and a few studies have observed ESBL-producing *Escherichia coli* in chicken meat, which may be associated with infection [1]. To date, no study has examined the wider commensal resistome associated with retail meat, which is vital when considering the potential of mobile resistance genes to transfer into the human microflora and pathogens. In this study, we tested the hypothesis that retail meat has a hidden reservoir of resistance genes in the animals' wider commensal flora.

Nine swab samples (three per carcass) were taken in March 2011 from retail organic chicken (UK reared), conventional chicken (UK reared) and budget chicken (source unavailable). Swabs were analysed for antibiotic resistance load using Luria broth agar plates supplemented with either ampicillin (32 mg/L), ciprofloxacin (1 mg/L),

cefotaxime (2 mg/L), chloramphenicol (16 mg/L), erythromycin (4 mg/L), gentamicin (4 mg/L), imipenem (8 mg/L), streptomycin (16 mg/L), sulfachloropyridazine (8 mg/L) or trimethoprim (4 mg/L). Swabs were also analysed using chromogenic medium (ChromoCult®; Merck Millipore, Feltham, UK) supplemented with the same antibiotics at clinical British Society for Antimicrobial Chemotherapy (BSAC) breakpoints [2]. Counts indicated that for all Gram-negative bacteria there were $>10^5$ CFU/swab resistant to six different antibiotics of five different classes (including 3GCs) (Fig. 1). When all Gram-positive and Gram-negative bacteria were considered, retail meat was found to contain $>10^6$ CFU/swab resistant to seven antibiotics covering five classes including 3GCs. Resistance to 3GCs is of particular concern and indeed third- and fourth-generation cephalosporins are not permitted for use in the UK in line with the Red Tractor Assurance scheme (http://assurance.redtractor.org.uk/resources/000/965/989/Poultry_Scheme_-_Broilers_and_Poussin_Standards.pdf; accessed 6 November 2014). To further investigate resistance to 3GCs, 60 Gram-negative 3GC-resistant isolates were purified from across the three chickens for analysis of mobile genetic elements and resistance genes. PCR screening for class 1 integrons and insertion elements IS*CR1* and IS*Ecp1*, which have previously mobilised resistance genes from the environment into clinical bacteria, allowed for the calculation of the number of resistance determinants per swab. A total of 60% of isolates were positive for integrons and 6.7% of isolates were positive for IS*CR1* and IS*Ecp1*; it was calculated that the number of 3GC-resistant bacteria per swab containing integrons was $3.8 \times 10^4$ and the number containing mobile elements IS*CR1* and IS*Ecp1* was $4.2 \times 10^3$. Screening for the common ESBLs $bla_{TEM}$, $bla_{SHV}$ and $bla_{CTX\text{-}M}$ [2] revealed that the prevalence of $bla_{CTX\text{-}M}$ was 11.3%, with 53.4% of isolates associated with a full

IS*Ecp1* module confirming gene mobility [2]. A total of 81.6% of isolates carried $bla_{TEM}$ and 5% carried $bla_{SHV}$. It can be calculated that there were $7.2 \times 10^3$ $bla_{CTX\text{-}M}$-containing 3GC-resistant bacteria per swab of chicken. Identification of the 3GC-resistant bacteria revealed that 82.8% of isolates were *Rahnella aquatilis*, an environmental bacterium [3], with other bacteria identified as *Serratia* sp. (5%) and *Pseudomonas* sp. (12.2 %).

This is the first report of $bla_{CTX\text{-}M}$ in *R. aquatilis* (sequencing revealed the genotype to be $bla_{CTX\text{-}M\text{-}3}$) and is cause for concern due to the large number present (>7000 per swab), which is higher than that found per millilitre of sewage-contaminated river sediment [2]. The finding of $bla_{CTX\text{-}M\text{-}3}$ is surprising as it is not commonly associated with human infection in the UK and is more common in Poland, where it is one of the most prevalent ESBLs, as well as in China [3]. This study illustrates the importance of screening a wide range of commensal and common environmental bacteria for carriage of resistance genes. *Rahnella aquatilis* is a common gut commensal in poultry, thus selection may have taken place in the animal gut on the farm. The bacterium can cause sepsis in immunocompromised patients [4], and indeed even when not causing infection the vast number of mobile genetic elements and resistance genes contained in the commensal flora of retail meat will be able to transfer to the human microbiome and enteric pathogens. We hypothesise that the resistant commensal flora of meat contributes to the abundance of diverse resistance genes often observed in human microbiome studies [5]. Clearly the use of indicator organisms such as *E. coli* is important for monitoring food quality, however when studying antibiotic resistance this study suggests we should consider the whole chicken microbiome or bacterial groups rather than single species, as focusing

on one bacteria alone may mask the underlying problem. If we consider that the most likely reason for exposure is through inadequately prepared food and improper hygiene, the same reason for food-related illness, and that the numbers of resistant bacteria and resistance genes present in the chicken microflora ($>10^5$) are much higher than the number required for infection by, for example, *Campylobacter* sp. ($>10^3$), then we can use the number of foodborne illnesses from chicken as measured by the Health Protection Agency as a proxy for exposure (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/246760/0174.pdf; accessed 21 November 2014). There are 398 420 cases of food poisoning a year from chicken in the UK; at least this number of people are exposed to the hidden resistome of retail chicken meat per year and the actual number could be several magnitudes greater due to such a large quantity of resistant commensals present in meat.

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## References

[1] Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect Dis 2013;13:155–65.

[2] Amos GC, Hawkey PM, Gaze WH, Wellington EM. Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. J. Antimicrob Chemother 2014;69:1785–91.

[3] Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother 2009;64(Suppl 1):i3–10.

[4] Chang CL, Jeong J, Shin JH, Lee EY, Son HC. *Rahnella aquatilis* sepsis in an immunocompetent adult. J Clin Microbiol 1999;37:4161–2.

[5] Sommer MO, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 2009;325:1128–31.

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**Fig. 1.** Number of antibiotic-resistant bacteria based on nine replicate swabs from across three chicken carcasses: (A) total number of bacteria; and (B) number of Gram-negative bacteria.

**Edited Figure 1**